CLAIMS

- 1. A method for direct transformation of a host cell comprising the steps:
- (a) generating partially overlapping intermediate fragments by polymerase chain reaction, said partially overlapping intermediate fragments further comprising a first intermediate fragment and a second intermediate fragment, said first and second intermediate fragments each comprising at least one mutated codon of interest, a flanking nucleotide sequence and a digestion site.
- (b) joining ends of said intermediate fragments to produce a linear product by fusion polymerase chain reaction;
 - (c) ligating of the linear product to create a circular product; and
 - (d) incubating said host cell with said circular product.
- 2. The method of claim 1 wherein said intermediate fragment containing said codon of interest comprises a forward and a reverse mutagenic primer comprising a desired mutation and a flanking sequence.
 - 3. The method of claim 1 wherein said digestion site is an Apal digestion site.
- 4. The method of claim 3 wherein said forward digestion site primers comprises the polynucleotide sequence GTGTGTGGGCCCATCAGTCTCACGACC.
- 5. The method of claim 3 wherein said reverse digestion site primers comprises the polynucleotide sequence GTGTGTGGGCCCTATTCGGATATTGAG.
 - 6. A vector for direct transformation of a host cell comprising
 - (a) forward mutagenic primer;
- (b) a reverse mutagenic primer, wherein said forward and reverse mutagenic primers have an overlapping portion upstream and downstream of said mutagenic codon of interest;
 - (c) a forward digestion site primer;
- (d) a reverse digestion site primer, wherein said forward and reverse digestion site primers each have a digestion site, said digestion sites fused at end to form a circular polynucleotide sequence.

- 7. The vector of claim 6 wherein said forward digestion site primer comprises the polynucleotide sequence GTGTGTGGGCCCATCAGTCTCACGACC.
- 8. The method of claim 3 wherein said reverse digestion site primer comprises the polynucleotide sequence GTGTGTGGGCCCTATTCGGATATTGAG.